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Bidirectional Myoblast-Pericyte Plasticity

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Microvascular pericytes are able to generate multiple mesenchymal cell types, including skeletal muscle myoblasts. Cappellari et al. (2013) report in this issue of *Developmental Cell* that myoblasts can return the favor by generating pericytes via the action of Dll4 Notch ligand and PDGF-BB.

Pericytes are the microvascular mural cell counterparts of smooth muscle cells in larger vessels, partnering with vascular endothelial cells during vessel morphogenesis and maturation. Extensive crosstalk occurs between pericytes and endothelial cells throughout all phases of vessel development, mediating not only initial interactions between the two cell types during vessel formation, but also maturation, stabilization, and remodeling during later stages of vessel morphogenesis. Multiple factors are involved in this crosstalk. For example, endothelial cells signal to pericytes via production of PDGF-BB and Notch ligands, among other factors, while pericyte-derived angiopoetin-1 and TGFβ are important signaling entities for endothelial cells (Armulik et al., 2005). The specificity and temporal significance of these various signals are still subjects of active investigation.

Pericytes exhibit tremendous plasticity in both their differentiation potential and their developmental origins (Armulik et al., 2011). The mesenchymal stem cell nature of pericytes (Caplan, 2008) is highlighted by several recent papers demonstrating the ability of pericytes to give rise to a variety of mesenchymal cell types in different organs, including skeletal muscle, smooth muscle, bone, cartilage, and adipose tissue (Brachvogel et al., 2005; Crisan et al., 2008). Developmentally, pericytes can arise not only from stem cell sources such as the bone marrow, but also from cells that reside in mature tissues such as adipose deposits and both skeletal and smooth muscles (Rajantie et al., 2004; Traktuev et al., 2008). The laboratory of Giullio Cossu has contributed to both sides of the pericyte story. Previously they demonstrated that pericytes in skeletal muscle could serve as myogenic precursor cells, distinct from but in parallel with the activities of more well-studied skeletal muscle satellite cells (Dellavalle et al., 2007). This group now reports, in this issue of *Developmental Cell,* the ability of vascular endothelial cells to convert skeletal muscle myoblasts into pericytes (Cappellari et al., 2013), emphasizing the bidirectional plasticity that exists within mesenchymal lineages.

Cossu's group noticed that when GFP-tagged embryonic mouse myoblasts (embryonic day 11.5) were cultured with human umbilical vein endothelial cells (HUVECs), the myoblasts began to express the pericyte marker alkaline phosphatase. Replacement of HUVECs with the endothelial cell products PDGF-BB and Notch ligand Dll4 led to complete inhibition of myoblast fusion, retention of proliferative ability, and upregulation of several pericyte markers, including alkaline phosphatase, RGS5, and NG2. While PDGF-BB alone was unable to produce these effects, Dll4 proved to be active in producing a pericyte phenotype (although with reduced potency) even in the absence of PDGF-BB and could not be replaced by other Notch ligands such as Dll1 or Jagged1. In addition, inhibition of Notch signaling blocked the expression of pericyte markers and allowed rapid myoblast fusion. Later-stage myoblasts (embryonic day 16.5) express the Myf5 transcription factor, indicative of greater commitment to a myogenic fate. Even at this later stage, however, treatment of myoblasts with Dll4 and PDGF-BB was still able to block myoblast fusion and induce pericyte marker expression. Thus, the phenotypic switch from myoblast to pericyte is not restricted to very early-stage myoblasts, but can still be induced at fairly advanced stages of myoblast commitment.

These observations raised the question of whether Dll4/PDGF-BB treatment of myoblasts produced a functional pericyte phenotype in addition to the marker phenotype. Pericytes essentially are defined by their perivascular location and by their ability to collaborate with endothelial cells in forming functional vascular tubes. When cultured in Matrigel with HUVECs, GFP-positive Dll4/PDGF-BB-treated myoblasts closely associated with the endothelial cells to form vessel-like networks that were stable for up to 2 weeks. This behavior mimicked that of bona fide pericytes, but was not reproduced by untreated myoblasts, which failed to stabilize endothelial networks. Moreover, subcutaneous implantation of Matrigel plugs containing HUVECs and Dll4/PDGF-BB-treated GFP-positive cells led to the formation in the plug of functional vasculature composed of the HUVECs and myoblast-derived pericytes. This process could be blocked by silencing HUVEC expression of Dll4 via shRNA treatment, showing that, while pretreatment of myoblasts with Dll4 was required to produce the angiogenic effect, HUVEC-derived Dll4 was also required for stabilizing and maintaining the pericyte/endothelial cell relationship in vivo.

In establishing the in vivo relevance of these findings, the Cossu group also demonstrated the presence of myogenic markers in rare perivascular cells, suggesting the possibility that pericytes may occasionally be derived from myoblasts during normal development. Pursuing this further, they developed a mouse model that allowed them to test the effects of increased Notch signaling on (1) myoblast generation of myotubes and

(2) myoblast conversion to a pericyte phenotype. The model utilized Rosa^{NICD} transgenic mice that express the Notch Intracellular Domain (NICD) following Cre-mediated recombination. Crossing these mice with MyoD^{Cre} partners thus activated Notch signaling specifically in MyoD-expressing myoblasts. Although some indications of an altered phenotype in mutant embryos were seen at embryonic day 11.5, obvious changes were not evident until embryonic day 13.5. Numbers and/or size of myosin heavy chain (MyHC)-positive myofibers were significantly reduced in mutant embryos at this stage, and perivascular cells expressing both Myf5 and alkaline phosphatase were easily detectable in mutant, but not control, embryos. By embryonic day 16.5, the altered phenotype was much more prominent in mutant embryos. Skeletal muscle development was severely reduced, while the pericyte markers PDGFRβ and alkaline phosphatase were clearly upregulated in perivascular cells. In addition, elevated Dll4 expression was observed in association with blood vessels, consistent with its proposed role in myoblast conversion to pericytes. The condition of mutant embryos continued to deteriorate at embryonic day 18.5, and the animals died at birth. Beyond their reduced skeletal muscle development, mutant embryos exhibited prominent edema. One possible explanation for this could be impaired vascular function (increased vessel leakiness). Thus, even though myoblast-derived pericytes appear capable of competing with normally derived pericytes for perivascular localization, they nevertheless may not fully mimic the functional properties of pericytes derived from normal developmental sources. It is intriguing to wonder whether the function of myoblast-derived pericytes is influenced by retention of certain aspects of their myogenic identity (for example continued expression of Myf5). Additional studies of vascular function, including evidence of increased hemorrhage, would have been a valuable addition to this work.

The authors suggest that the observed mechanisms may be relevant to myoblasts located at the boundary between myogenic and angiogenic tissues, where the balance of myogenic versus angiogenic factors determines their developmental fate. Under normal conditions, this balance appears to be heavily weighted toward a myogenic fate, as evidenced by the scarcity of myoblast-derived perivascular cells. However, in pathological situations that require the formation of new blood vessels, the balance may swing toward an angiogenic fate under the influence of factors produced by activated vascular endothelial cells. This story highlights the bidirectional developmental plasticity of mesenchymal cells because Cossu and colleagues have previously reported the ability of pericytes to generate myoblasts (Dellavalle et al., 2007). This group has also demonstrated the potential therapeutic utility of pericyte transplantation for treating muscle disorders (Tedesco and Cossu, 2012). At this stage, it is unclear to what extent myoblast transplantation could be an effective strategy for proangiogenic therapy.

REFERENCES

Armulik, A., Abramsson, A., and Betsholtz, C. (2005). Circ. Res. *97*, 512–523.

Armulik, A., Genové, G., and Betsholtz, C. (2011). Dev. Cell *21*, 193–215.

Brachvogel, B., Moch, H., Pausch, F., Schlötzer-Schrehardt, U., Hofmann, C., Hallmann, R., von der Mark, K., Winkler, T., and Pöschl, E. (2005). Development *132*, 2657–2668.

Caplan, A.I. (2008). Cell Stem Cell *3*, 229–230.

Cappellari, O., Benedetti, S., Innocenzi, A., Tedesco, F.S., Moreno-Fortuny, A., Ugarte, G., Lampugnani, M.G., Messina, G., and Cossu, G. (2013). Dev. Cell *24*, this issue, 586–599.

Crisan, M., Huard, J., Zheng, B., Sun, B., Yap, S., Logar, A., Giacobino, J.P., Casteilla, L., and Peault, B. (2008). Curr. Protoc. Stem Cell Biol. http://dx.doi.org/10.1002/9780470151808.sc02b02s4, Chapter 2: Unit 2B.2.1–2B.2.13.

Dellavalle, A., Sampaolesi, M., Tonlorenzi, R., Tagliafico, E., Sacchetti, B., Perani, L., Innocenzi, A., Galvez, B.G., Messina, G., Morosetti, R., et al. (2007). Nat. Cell Biol. *9*, 255–267.

Rajantie, I., Ilmonen, M., Alminaite, A., Ozerdem, U., Alitalo, K., and Salven, P. (2004). Blood *104*, 2084–2086.

Tedesco, F.S., and Cossu, G. (2012). Curr. Opin. Neurol. *25*, 597–603.

Traktuev, D.O., Merfeld-Clauss, S., Li, J., Kolonin, M., Arap, W., Pasqualini, R., Johnstone, B.H., and March, K.L. (2008). Circ. Res. *102*, 77–85.